

## Annexin A1 (ANXA1): A Systematic Review of Its Role in Inflammation (Aneksin A1 (ANXA1): Suatu Ulasan Sistematis tentang Peranannya dalam Keradangan)

SANGGAVI NADARAJAN, NORAZRINA AZMI, MALINA JASAMAI & ENDANG KUMOLOASASI\*

### ABSTRACT

*Inflammation is a body response towards any injury or tissue damage. It involves the accumulation of neutrophils and release of inflammatory mediators. ANXA1, a 37 kDa glucocorticoid inducible protein plays an important role in resolving inflammation. The unique N-terminal and its mimetic peptide exert strong anti-inflammatory actions. This study was conducted to review the roles of ANXA1 in inflammation and identify any other reported roles. Electronic search was done whereby a total of 3797 articles were located from three databases, namely Ovid MEDLINE, Science Direct, and PubMed. Articles on ANXA1 and inflammation were selected based on inclusive criteria and review papers were excluded. Bias analysis was performed based on bias risk tool and 27 articles were included in the study. It was found that ANXA1 was able to resolve inflammation in many inflammatory diseases. Upon treatment with glucocorticoid, ANXA1 is induced and its significant expressions in tissues are important in resolving inflammation. However, this effect can be reversed by administering an anti-annexin antibody. This protein also acts on members of all formyl peptide receptors (FPR) and activates them to initiate reaction. It acts mainly by causing the death of neutrophils through apoptosis. In addition, ANXA1 is identified as a marker in cancer cells which determines the survival rates. In conclusion, ANXA1 is a key modulator in resolving inflammation in many diseases and is actively being induced upon glucocorticoid treatment.*

*Keywords: Annexin A1; Annexin I; ANXA1; inflammation; Lipocortin-1; phospholipase A2 inhibitory protein*

### ABSTRAK

*Keradangan adalah tindak balas badan terhadap sebarang kecederaan atau kerosakan tisu. Ia melibatkan pengumpulan neutrofil dan pembebasan perantara keradangan. ANXA1, sejenis protein yang boleh diaruh glukokortikoid 37 kDa memainkan peranan penting dalam menangani keradangan. Terminal-N yang unik dan peptida mimetiknya menunjukkan tindakan anti-keradangan yang kuat. Kajian ini dijalankan untuk mengkaji peranan ANXA1 dalam keradangan dan mengenal pasti lain-lain peranan yang dilaporkan. Pencarian elektronik dilakukan dan sejumlah 3797 artikel diperolehi daripada tiga pangkalan data iaitu Ovid MEDLINE, Science Direct dan PubMed. Artikel mengenai ANXA1 dan keradangan dipilih berdasarkan kriteria inklusif dan tidak termasuk artikel ulasan. Analisis pincang dilakukan berdasarkan peralatan risiko pincang dan 27 artikel dimasukkan dalam kajian. Didapati bahawa ANXA1 dapat menangani keradangan dalam pelbagai penyakit radang. Semasa rawatan menggunakan glukokortikoid, ANXA1 diaruh dan ekspresi ketaranya dalam tisu adalah penting dalam menangani keradangan. Walau bagaimanapun, kesan ini dapat dibalikkan dengan menggunakan antibodi anti-aneksin. Protein ini juga didapati bertindak pada semua jenis reseptor peptida formil (FPR) dan mengaktifkannya untuk memulakan tindak balas. Ia bertindak terutamanya dengan menyebabkan kematian neutrofil melalui apoptosis. Sebagai tambahan, ANXA1 dikenal pasti sebagai penanda sel barah yang menentukan kadar kemandirian. Kesimpulannya, ANXA1 adalah modulator utama dalam menangani keradangan dalam pelbagai penyakit dan secara aktif diaruh oleh rawatan glukokortikoid.*

*Kata kunci: Aneksin A1; Aneksin I; ANXA1; keradangan; Lipokortin-1; protein penyekat fosfolipase A2*

### INTRODUCTION

Inflammation is a process which reacts upon any damage or injury to tissues (Soehnlein & Lindbom 2010). Redness, swelling, pain, heat, and loss of function often denote inflammation. The process of inflammation involves a few important events, such as vasodilatation, vascular

leakage and expression of inflammatory mediators. Then, all these events will cause the build-up of neutrophils, macrophages and other inflammatory cells which migrate to the site of injury or infection. In inflammatory process, vascular endothelium plays a vital role as it releases eicosanoids and cytokines. These molecules are known to cause inflammation (Ricciotti & Fitzgerald 2011).

The function of vascular endothelium is to engage leukocytes via the presence of adherent molecule to the site of injury (Cronstein & Weissmann 1993). Inflammation causes the emergence of a scar (Ker et al. 2000). Resolution of inflammation is important in regulating our body balance (Ortega-Gómez et al. 2013). Death of neutrophil is an important event towards resolution of inflammation and it is engulfed by macrophages (Fox et al. 2010). The removal of neutrophil shifts the situation to anti-inflammatory condition (Fadok et al. 1998). At the final stage for neutrophil to undergo apoptosis, it releases mediators to block the neutrophil engagement during the inflammatory process (Ortega-Gómez et al. 2013).

One of the proteins released during inflammation is ANXA1 (Ortega-Gómez et al. 2013). ANXA1 or better known as lipocortin-1 (37 kDa) is a glucocorticoid inducible protein that plays certain biological functions (Gerke et al. 2005). It is mostly found in neutrophils, eosinophils, macrophages, mast cells and myeloid lineage (Lima et al. 2017). Anti-inflammatory actions are actually demonstrated by its unique N-terminal peptide. Peptide derived from ANXA1, which is Ac2-26, is able to mimic the action (Vong et al. 2007).

These effects are regulated upon its interaction via GPCR: FPR Type 2/lipoxin A<sub>4</sub> receptor (ALX). Moreover, it has different functions, such as cell proliferation, differentiation and membrane trafficking (Gerke et al. 2005). Its anti-inflammatory effects are seen through the blocking of the leukocyte movement, PLA<sub>2</sub>, COX-2, and iNOS expression. It also stimulates the release of IL-10 and is involved in neutrophil apoptosis (Lima et al. 2017). This protein is secreted whenever stress-induced inflammation takes place by rising the cortisol production (Probst-Cousin et al. 2002).

Several conducted studies showed that lack of ANXA1 could lead to etiology of inflammatory disease (Kosicka et al. 2013). This systematic review is mainly conducted to review the relation between ANXA1 and inflammation, especially its role in inflammatory diseases and determine its role in inflammatory process.

## MATERIALS AND METHODS

### SEARCH STRATEGY

This study was mainly conducted to review studies of ANXA1 in inflammation. Information for this study was assembled by using electronic bibliographic databases, such as Ovid MEDLINE, PubMed, and Science Direct (Lopresti 2017). To search for these articles, relevant subject headings and keywords were used, such as inflammation, redness, pain, swelling, annexin A1, ANXA1, lipocortin-1, annexin 1, and phospholipase A<sub>2</sub> inhibitory protein. Synonyms of the word 'inflammation'

and 'annexin A1' were also used to extend the search. Upon increasing the sensitivity of search, Boolean operator 'AND' was used to combine the keywords (Hatah et al. 2014). Examples of search terms used were 'inflammation OR redness OR pain OR swelling' AND 'annexin A1 OR ANXA1 OR lipocortin-1 OR annexin 1 OR phospholipase A<sub>2</sub> inhibitory protein'.

### INCLUSIVE AND EXCLUSIVE CRITERIA

**Inclusive criteria:** To select relevant articles, they were required to be in full report, which could be from all types of study design with a control or comparison group. To be accepted, the study in articles may involve annexin A1 and inflammation, as well as written in English. **Exclusive criteria:** Articles were excluded from this study if they were conference report, manual, patent, bibliography, review articles, symposium, book, bibliometric study, index, editorial or survey. Articles were also excluded if they were written in non-English. Then, the articles were imported into Reference Manager, and EndNote. Meanwhile, duplicates were removed (Aziz et al. 2016). Articles were also excluded if the abstract or journal was unattainable. Additional relevant studies were conducted by assessing the reference lists of included literature. These additional references were then compared with the original search results while duplicates were removed (OHAT 2015).

### STUDY SELECTION

Based on the inclusive and exclusive criteria, the retrieved articles were screened. Articles which describe a clear methods, results and outcome were included for analysis (Aziz et al. 2016). Titles or abstracts were screened. Articles that were not relevant to the research title were excluded. Next, full text articles were retrieved to select articles which have met the inclusion criteria. The final decision on article selection will depend on the full text (OHAT 2015).

### DATA EXTRACTION AND ANALYSIS

Data were extracted to evaluate the role of ANXA1 in inflammation by using data extracted from the Office of Health Assessment and Translation for conducting the systematic review, including evidence integration (OHAT 2015) which consisted of study design, experimental model, methods and results. Data were divided into *in vitro* and *in vivo* studies. Data were analysed by using a narrative review. The study analysis was focused more on the results obtained from the experiments in each study. The first author, year, animal models and treatment/compounds (*in vivo* studies), cell/ tissue model/cell type (*in vitro* studies), methods, results and comments were

included and a summary of the findings is shown in Tables 1 and 2 (supplemental data).

## RESULTS AND DISCUSSION

### BIAS ANALYSIS

Bias was analysed by using the bias risk tool provided by the Office of Health Assessment and Translation (OHAT) bias risk tool handbook. The potential bias of study was evaluated.

### SEARCH RESULTS

A total of 3797 articles were imported into the Endnote from three different databases. Taking into consideration the inclusion and exclusion criteria, a total of 27 articles were included in the final analysis (Figure 1). A summary of the findings is included in Tables 1 and 2 (Supplemental data).

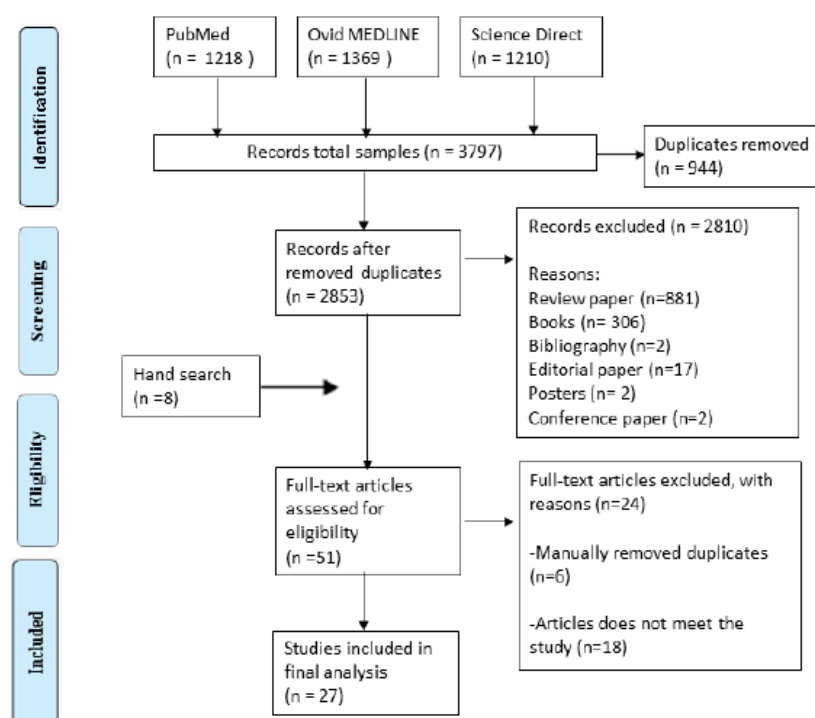


FIGURE 1. Flow diagram on inclusion and exclusion of systematic review

### ANXA1 IN INFLAMMATORY DISEASES

In autoimmune diseases, ANXA1 was found to restrict TH17 cells and attenuated the severity. Complete Freund's adjuvant (CFA) and human recombinant protein ANXA1 were administered into the ANXA1 deficient and wild type (WT) mice. Control mice were left untreated. The expression of annexin protein was detected by using western blot technique. Mice which did not have ANXA1 exhibited severe inflammation, which was significantly different ( $p < 0.05$ ) from the WT mice. Enhanced level of

monocytes, neutrophils, macrophages and Th17- CD4<sup>+</sup> T cells were observed in ANXA1 deficient mice. Absence of this protein lead to T-cell proliferation and expression of ANXA1 diminished the pro-inflammatory cytokines and autoimmune retinal inflammatory disease. Lack of ANXA1 expression showed an increase in inflammation severity.

This was because proliferation and activation of TH17 cells took place uncontrollably. These events occurred due to the small capability to introduce SOCS3, which gave an unrestrained phosphorylation

of STAT3 pathway. CD4<sup>+</sup> cells isolated from tissues that lack ANXA1 demonstrated a greater level of TH17 cells. Therefore, the delivery of human recombinant ANXA1 restricts CD4<sup>+</sup> cell proliferation and lowers pro-inflammatory cytokines, such as IL-17, IFN- $\gamma$ , IL-6, which reduces the severity of inflammation. Therefore, it showed that this protein had actively regulated the TH17 cells (Yazid et al. 2015).

In addition, another study was conducted to evaluate the beneficial effect of ANXA1 in experimental allergic conjunctivitis (AC). The experiment was carried out by giving ovalbumin injection to cause AC by using WT and ANXA1 knockout mice. Both types of mice were then treated with mimetic peptide Ac-26 and dexamethasone. Negative control mice from both groups were treated with normal saline. The p value of test groups indicated a statistically significantly different ( $p < 0.05$ ) as compared to the negative control group. Therapy by using ANXA1 mimetic peptide and dexamethasone had markedly lowered the clinical appearance of AC, IgE, leukocyte and mast cell degranulation in conjunctiva of ANXA1 knockout mice as compared to WT mice. Therefore, the effect of dexamethasone was associated with enhancement of ANXA1 expression in ocular tissue (Gimenes et al. 2015). The study of Rodrigo et al. (2004) demonstrated the expression of ANXA1 in normal and chronically inflamed nasal mucosa. Samples were obtained from healthy subjects, patients with perennial rhinitis and nasal polyps. This protein was found to be expressed highly in ciliated cells. However, the study had shown that there was no difference in expression between healthy and normal cel.

Another study was conducted on ANXA1 deficient and WT mice to determine the airway hyper-responsiveness in mouse model of asthma. Mice and control mice were given ovalbumin and normal saline, respectively. The lung compliance and airway resistant were determined in both types of mice. The level of IgE, IgG2a, and IgG2b were high in ANXA1 deficient mice and they were more sensitive towards airway responsiveness as compared to WT mice. This suggested that ANXA1 played a critical role in asthma as the absence of ANXA1 caused a rise in antibody response (IgE, IgG2a, IgG2b) and hypersensitivity in asthma (Ng et al. 2011).

Similar expression of ANXA1 was seen in ulcerative colitis (UC) patients when tissue samples from mild, moderate, severe UC of inflamed colons, and severe UC from noninflamed colons were collected. Before tissue samples were taken, patients were given amino salicylate (ASA) and oral prednisolone. Healthy patients without UC served as control. The release of ANXA1 was detected in inflamed severe UC patients but not detected in slight or moderate UC. Myeloperoxidase (MPO) activity was used to evaluate the extent of tissue granulocyte infiltration.

Therefore, the MPO activity in healthy colons was not statistically significantly different with  $p = 0.606$ , while  $p = 0.036$  for the inflamed colons as compared to the control subjects (Vergnolle et al. 2004). A study on gastric mucosal lesion by Martin et al. (2008) showed similar results, in which this protein had a vital role in regulating the damage. The role of ANXA1 was tested in ANXA1 deficient and wild type mice. After the induction of ulcer, mice were given ANXA1 mimetic peptide, Ac2-26 and dexamethasone. Healthy mice were kept as control subjects. Western blot was used to examine the presence of this protein during the curing of injury process. ANXA1 expression was strongly increased in ulcerated gastric tissue and administration of an ANXA1 mimetic peptide improved ulcer healing, whereas an antagonist of the main receptor for ANXA1 impaired healing. The ANXA1-deficient mice showed similar susceptibility to indomethacin-induced gastric damage. However, the healing of that damage was significantly impaired in the ANXA1-deficient mice as compared to the wild-type mice. The expression of ANXA1 was significantly different from healthy controls,  $p < 0.05$  (only found intact ANXA1, 37 kDa in the healthy mice, but in the ulcer cleavage product of ANXA1 33 kDa) was also found. Furthermore, ANXA1 was highly expressed in gastric mucosal lesion. Alleviation of gastric wound was achieved by administering ANXA1 mimetic peptide, Ac2-26. Since ANXA1 binds and activates FPR; hence, antagonist of FPR will reverse the process of healing. Boc2, the FPRL-1 as an antagonist of FPR also showed the corresponding outcome, whereby they impaired the healing. Not only this protein (ANXA1), but lipoxin A4 also promotes the reduction of inflammation (Martin et al. 2008). Taken together, these data support the hypothesis that ANXA1 makes an important contribution to the healing of gastric mucosal damage.

Investigation on the reduction of plasma ANXA1 was done in obese population. ANXA1 level was assessed in human plasma obtained from overweight and obese patients, whereas the normal subjects served as a control. There was an opposite correlation with  $p < 0.001$  between plasma ANXA1 protein and body mass index (BMI), as well as to that of waist to hip ratio (WHR). As the fat rises, the ANXA1 concentration decreases. Low level of ANXA1 was observed in high WHR. There was no relation between ANXA1 to blood glucose and age (Kosicka et al. 2013).

Another study was conducted on multiple sclerosis patients, whereby their tissues and those without any neurological diseases were collected to investigate the expression of ANXA1. Control cells were left untreated and ANXA1 was found to be expressed in multiple sclerosis lesion with reduction in concentration from active lesions to chronic levels. Multiple sclerosis (MS) patients had ANXA1 expression detected in the plaques which were in



macrophages and reactive astrocytes. ANXA1 abolishes the activation of T-cell lines. Increase in ANXA1 expression in active astrocytes leads to differentiation and activation of astrocytes. The additional presence of ANXA1 gives impact to lymphocytes and macrophages to reduce inflammation. In CNS patients of MS, the treatment upon glucocorticoids causes the induction of ANXA1 which contributes in decreasing the symptoms of inflammation (Probst-Cousin et al. 2002).

Reduction of ANXA1 concentration in human urothelium (UE) and cell line TEU-2 reduced the cell survival upon subjection to bacterial toxin. In the same study, cells were obtained from patients with no symptom and from bladder pain syndrome (BPS) patients. The distribution of ANXA1 was mainly on cytoplasm and mRNA gene expression was reduced in BPS patients as compared to control patients (no symptom) subjects ( $p < 0.05$ ). This study showed that there was a decrease in cell survival in absence of ANXA1 (Monastyrskaya et al. 2013).

#### THE DISTRIBUTION AND LOCALISATION PATTERN OF ANXA1

Complete Freund's adjuvant (CFA) administration caused chronic inflammation in mice. Tissue samples were obtained from healthy and inflammatory mice. It was then used for ANXA1 and mRNA gene detection. The result showed that two different bands of ANXA1 were formed, which are the active form 37 kDa and inactive form 33 kDa. ANXA1 was detected in neutrophils, macrophages, eosinophils, and fibroblasts. The level of ANXA1 was high after CFA injection and significantly different from untreated mice ( $p < 0.01$ ). The distribution pattern was mainly observed in cytoplasm and cell surface (Gibbs et al. 2002). Administration of carrageenin had increased ANXA1 level in cytoplasm and administration of anti-ANXA1 antibody detected ANXA1 in nucleus and cytosol. There was a distribution of ANXA1 in matrix and granules of the cytosol (Oliani et al. 2002).

#### ANNEXIN A1 CAUSES THE LEUKOCYTE EMIGRATION AND NEUTROPHIL DETACHMENT

Human leukocyte elastase (HLE) caused the cleavage of ANXA1 brimful length of 37 kDa protein into smaller part, which is inactive 33 kDa protein. Upon endothelial fixing, ANXA1 was detached from the leukocyte and cleaved. Administration of ANXA1 to leukocytes caused the shortening of ANXA1, which was influenced by the HLE activity. The sustained ANXA1 at the leukocyte has a role in controlling leukocyte emigration via its N-terminal peptide into the damaged tissue at the inflammatory place (Rescher et al. 2006).

ANXA1 and its mimetic peptide are thought to cause neutrophil detachment to control inflammatory process. This was proven by conducting an experiment on mice, whereby zymosan was injected and neutrophil adherent to post capillary venule was assessed. ANXA1 and its mimetic peptide were then injected into the mice. Control animals were treated with normal saline. Delivery of zymosan caused a rise in cell adhesion and emigration, but once ANXA1 and mimetic peptide were given, it caused a drastic detachment of neutrophil. The leukocytes were separated and returned to the bloodstream. This result was significantly different from the control group ( $p < 0.05$ ) (Lim et al. 1998).

ANXA1 blocks leukocyte movement through reduction of neutrophils and monocytes adhesion to vascular endothelium (Ferlazzo et al. 2003), thus, causes the death of neutrophils (Monastyrskaya et al. 2013). In inflammatory condition, neutrophils permeate to sites of inflammation (Rodrigo et al. 2004) and this permeation is determined by the expression of ANXA1 (Gibbs et al. 2002). The amount of neutrophils increased upon inflammation, whereby ANXA1 controlled their accumulation (Gibbs et al. 2002). Administration of ANXA1 mimetic peptide blocks the engagement of neutrophils in acute and chronic inflammation. ANXA1 lowers neutrophil adhesion and migration, and thus develops the separation of neutrophils adherent to post capillary venules to regulate inflammation (Lim et al. 1998).

#### STIMULATION OF IL-10, BLOCKS THE NITRIC OXIDE SYNTHESIS

Murine macrophages cell line J774 were collected and cultured. The release of nitric oxide (NO) was determined. The cells were then induced with LPS and the control cells were without induction. Then, RNA was isolated from treated and control cells. It was found that LPS caused the release of IL-10 (anti-inflammatory cytokine). ANXA1 peptide was also able to induce IL-10 and block the NO liberation. Peptide Ac2-26 as mimic of ANXA1 was able to block nitric oxide synthase (iNOS) and its mRNA gene accumulation. Therefore, role of ANXA1 as an anti-inflammatory mediator has caused the induction of IL-10, which then blocked the nitric oxide synthesis (Ferlazzo et al. 2003). NO was produced due to inflammation and from the role of iNOS in cells such as macrophages, synovial fibroblast, osteoblasts, and chondrocytes. The release of NO was mediated by pro-inflammatory cytokines. ANXA1 acted in this situation by moving to the cell surface upon treatment of dexamethasone. One of the actions of ANXA1 is that it blocked the NO after it was inducted by glucocorticoids (Yang et al. 1998). Dexamethasone was able also to

inhibit hyperalgesic effect produced by PGE<sub>2</sub>, carrageenin, bradykinin, TNF- $\alpha$ , IL-6, IL-8 and dopamine (Ferreira et al. 1997). ANXA1 was able to inhibit the hyperalgesic effect by blocking the cytokines and the COX-2 which produced prostaglandin. However, the addition of anti-ANXA1 antibody reversed the inhibitory action (Ferreira et al. 1997). Ac2-26 was able to regulate neutrophilic inflammation by stimulating route of Bax, casapase 3 and blocking the survival pathways, such as Mcl-1, extracellular signal-regulated Kinase-1 (ERK1/2) and NF- $\kappa$ B (Vago et al. 2012).

#### ANXA1 AND FORMYL PEPTIDE RECEPTOR

Peripheral blood granulocytes, monocytes, and HEK 293 cells express formyl peptide receptor (FPR), formyl peptide receptor like-1 (FRP 1), and formyl peptide receptor like 2 (FPR 2) were used. Proteolytic enzymes cleavage the N-terminal domain of ANXA1 produced Ac1-25 peptide. This peptide changed the neutrophil morphology and ANXA1 was able to activate all FPR family at the monocytes and granulocytes. However, it was clear that the activation of FPRs had more complex consequences and could also promote the resolution of inflammation. It was found that one of the anti-inflammatory actions of this protein was due to activation of FPR (Ernst et al. 2004). Moreover, this protein also exerted its anti-nociceptive effects via FPR. When mice were induced with CFA to cause inflammation, BML-111, Boc-1 and Anxa 1<sub>2-26</sub> were injected. Control mice group was left untreated. The administration of CFA caused increase in ANXA1 significantly with  $p < 0.01$  as compared to control mice. BML-111 and Anxa 1<sub>2-26</sub> decreased the mechanical nociception, whereas Boc-1 reversed the action (Pei et al. 2011).

#### ROLE OF ANXA1 IN INFLAMMATION VIA INHIBITION OF INFLAMMATORY MEDIATORS

Arachidonic acid is synthesised from linoleic acid and produces PLA<sub>2</sub>. This acid functions as a substrate for a few vital enzymes, in which some of them are cyclooxygenase to produce prostaglandin and lipoxygenase for formation of hydroperoxyicosatetranoic acid. These enzymes are found to be responsible for inflammation. Therefore, ANXA1 acts to inhibit these enzymes in order to resolve inflammation (Hirata et al. 1980). It also blocks the eicosanoid mediators, interrupts leukocyte diapedesis by attaching to specific surface receptors of granulocytes and macrophages (Flower & Rothwell 1994). ANXA1 can block the COX-2 through administration of dexamethasone and stops the release of prostaglandin. This eventually leads to inhibition of hyperalgesic effects (Ferreira et al. 1997). In non-small cell lung cancer cells, the expression of COX-2 is high,

thus, promotes to inflammatory status. Green tea extract (GTE) is found to stimulate the release of ANXA1 in cancer cells so as to help reduce inflammation by inhibiting COX-2. Therefore, stopping further release of PGE<sub>2</sub> (Lu et al. 2012). IL-1 $\beta$  has enhanced the presence of COX-2. GTE can block COX-2 and PLA<sub>2</sub> via stimulation of ANXA1. GTE influences in a few inflammatory routes through regulation of NF- $\kappa$ B, epidermal growth factor receptor (EGFR) or human epidermal growth factor receptor 2 (HER2) and mitogen-activated protein kinase (MAPK) (Adhami et al. 2007).

#### ANXA1 AS A MEDIATOR OF GLUCOCORTICOID ACTION

A study by Hirata et al. (1980) used rabbit peritoneal neutrophils treated with glucocorticoids. Control cells were left untreated. This study was conducted to determine the inhibition of PLA<sub>2</sub> pathway, upon release by arachidonic acid and inhibited by PLA<sub>2</sub> inhibitory protein. It provided evidence that when glucocorticoids were given, it stimulated the release of ANXA1, which then lowered the synthesis of prostaglandins by blocking PLA<sub>2</sub> pathway. Percentage of inhibition varied, depending on the strength of glucocorticoids used. Flucinolone acetone had the highest inhibition and this action was regulated by glucocorticoid receptors. To determine this protein's action on glucocorticoid receptor, LPS induced mice were treated with dexamethasone and rolipram. Peptide Ac2-26 was given as mimic peptide of the ANXA1 action. Administration of rolipram and dexamethasone lowered the number of neutrophils and enhanced cell death. The level of ANXA1 was significantly high ( $p < 0.01$ ) as compared to the control mice. There was a correlation between intact ANXA1 and proapoptotic events. Stimulation effect of Ac2-26 due to glucocorticoid treatment helped to resolve inflammation by providing neutrophil cell death (Vago et al. 2012). A similar study showed that upon administration of dexamethasone, ANXA1 was induced. When rats were given PGE<sub>2</sub>, carrageenin, bradykinin, and dopamine, it evoked hyperalgesic actions. However, this action was blocked significantly by the administration of ANXA1 as compared to untreated rats. Therefore, it indicated that the anti-hyperalgesic actions of dexamethasone was performed through ANXA1 role (Ferreira et al. 1997).

Synovial tissues of adjuvant arthritis (AA) induced rats were removed and treated with LPS and dexamethasone. Control rats were treated with purified mouse IgG. Due to the presence of glucocorticoid, ANXA1 was induced and found to be expressed in synovial macrophages in a high number. Dexamethasone inhibited significantly ( $p < 0.001$ ) with NO production from control rat. A study by Yang et al. (1998) also supported the role of ANXA1 in blocking NO production upon

glucocorticoid treatment. When topical glucocorticoid was given, the same result indicated that it was induced by ANXA1. Rats treated with betamethasone-17-valerate and control rats with vehicle and the presence of ANXA1 was detected. This glucocorticoid inhibited oedema formation at 55% with p-value of <0.01 as compared to vehicle control. Glucocorticoids interrupt inflammation and act as immunosuppressants. They are used to treat inflammatory diseases, mainly autoimmune disorders. However, its mechanism is not fully understood but is found to depend on regulatory proteins (Vago et al. 2012). Induction of this protein (ANXA1) by glucocorticoid is controlled through the cytoplasmic glucocorticoid receptors but can decline via the addition of antagonist such as progesterone (Hirata et al. 1980). ANXA1 was also induced during the administration of glucocorticoids in inflammatory arthritis. Mice which received arthritogenic K/BxN as an arthritis model were given dexamethasone to treat inflammation and control mice were left untreated. The tissue from mice was taken for analysing the ANXA1 concentration. Results showed that the presence of dexamethasone increased ANXA1 significantly with  $p < 0.05$  as compared to controlled mice. Therefore, ANXA1 acts as an indicator to determine the effectiveness of dexamethasone in inflammatory arthritis (Patel et al. 2012). Glucocorticoids block the phospholipase A2 in affecting the conversion of arachidonic acid to prostaglandin. ANXA1 is also inhibitor for PLA2 and its synthesis is induced by glucocorticoid. This study proposed that ANXA1 inhibits the chemotaxis of neutrophils by blocking the phospholipase A2. Through this, it regulates the lymphocyte mitogenesis, release of histamine from mast cell, chemotaxis of leukocyte, function of bradykinin on fibroblast and desensitisation of  $\beta$ -adrenergic receptors on C6 astrocytoma cells (Hirata et al. 1980). Therefore, from these studies it can be concluded that the anti-inflammatory effect was produced via induction of ANXA1 by glucocorticoid (Ahluwalia et al. 1995).

#### ANXA1 ACTING AS A STRESS PROTEIN

To determine whether ANXA1 is a stress protein, A549 and HeLa cells were treated with heat, hydrogen peroxide ( $H_2O_2$ ) and sodium arsenite and control cells were left untreated. Upon heat,  $H_2O_2$  and sodium arsenite treatment, ANXA1 and Mrna gene were stimulated, released and the level increased in both cell types. Besides, this protein was dispersed from cytoplasm to nucleus in untreated cells, whereas to nucleus and perinuclear region in treated cells. This movement indicated that it is present in nucleus once the treated cells were under stress condition. Its role in resolving stress induced transcriptional activation was investigated and the alteration was significantly different from untreated cells ( $p < 0.05$ ) (Rhee et al. 2000).

#### EXPRESSION OF ANXA1 IN CANCER CELL PROGRESSION

A study conducted on isolation of phospholipase A2 (PLA2) inhibitory protein (ANXA1) which is a prognostic influence on oral squamous cell carcinoma. An immunohistochemical procedure was carried out to determine the expression of ANXA1 as a marker in predicting the prognosis of oral cancer. Experiment was conducted by using specimens from healthy patients as control, oral epithelial dysplasia (OED) and oral squamous cell carcinoma (OSCC). In normal oral epithelia (NOE), this protein was found significant in plasma membrane but reduced in OED and OSCC (Lin et al. 2008). Its presence of ANXA1 in nucleus was significant for OED and OSCC. Enhancement in ANXA1 expression linked with bigger tumour size and distant metastasis. ANXA1 nuclear pigmentation in tumour and distant metastasis had the poorest survival rates. The lowering of ANXA1 in membranous pigmentation in OSCC was not significantly linked to patient's overall survival rate. ANXA1 nuclear positive tumours had lower survival rates than that of nuclear negative tumours. It showed that ANXA1 nuclear staining predicted a poor overall survival. In cancer cells, ANXA1 is mainly localised in nuclear and directly participates in tumour invasion. The nuclear translocation took part in controlling cell proliferation. Therefore, this protein is an important marker to speculate the prognosis of oral cancer (Lin et al. 2008).

In another study, they ruled out to investigate the effects of ANXA1 mimetic peptide in cervical cancer. SiHa cells obtained from cervix were treated with ANXA1 mimetic Ac2-26. Control cells were left untreated. From this study, the cell proliferation, gene expression and morphology were identified. Upon treatment with Ac2-26, there were no morphological changes. This mimetic peptide has shown to reduce cell proliferation of cancer cells as compared to the untreated group with  $p < 0.05$ . Five genes, which were TPT1, LDHA, NCOA3, HIF1A, and RAB13, were upregulated and one was down regulated which is the ID1 gene (Prates et al. 2015). From this study, ANXA1 may regulate ID1 gene by decreasing its expression and may be used as a possible therapeutic target. ID1 belongs to a family of small molecules of short length (13 to 20 kDa) and acts as an inhibitor of differentiation or DNA binding and it also regulates the effects of transcription factors on targeted genes. This gene was over expressed in cervical cancer but after treatment with peptide, it was reduced. Over expression lead to antagonistic of tumour cells and became independent marker for this tumour. When ANXA1 was present at low level in cervical cancer, it could be used as a marker in tumour progression. From this study, the involvement of ANXA1, Ac2-26 in the altered expression of genes involved in tumorigenic processes, which could potentially be applied as a therapeutic indicator of cervical cancer (Prates et al. 2015).

Studies on impact of green tea in inducing ANXA1 have shown to block the COX pathway in non-small cell lung cancer cells (NSCLCC). Cells of A549, H157, and H460 of lung cancer were treated with green tea extract (GTE) and control group was left untreated. Green tea has enhanced the level of ANXA1 protein and ANXA1 mRNA expression in H157 and H460 cells. Induction of ANXA1 by GTE caused the blockage of COX-2 expression in all three cells. However, the presence of IL-1 beta has caused reduction in GTE induced ANXA1. Rise in the level of ANXA1 has caused reduction in the COX-2 and PLA2 in A549 and H460 cells. Up regulation of COX-2 has led to enhance production of prostaglandin. This in turn caused an increase in cell proliferation. Blocking COX 2 and PLA2 activity of ANXA1 has been influenced by types of cell used. GTE is efficacious in inducing ANXA1; hence, inhibiting COX-2 in non-small cell lung cancer cells (NSCLCC) (Lu et al. 2012). Gao et al. (2014) studied about ANXA1 expression profile in noncancerous human gastrointestinal tissues by using RT-PCR ANXA1 was expressed in all investigated healthy tissues but the amounts varied, whereby liver showed the highest mRNA and decreased, followed by the colon, stomach, esophagus, pancreas, and bile duct, respectively. Cyclooxygenase-2 (COX-2) is an inducible enzyme which accumulates in activated macrophages and other cells at sites of inflammation. Increasing evidences showed that COX-2 was upregulated in various carcinomas and played a key role in tumorigenesis (Gao et al. 2014). Furthermore, this study also compared the expression of ANXA1 and COX-2 between noncancerous and cancerous cell, and it found that COX-2 expression in these gastric cancer cases correlated with ANXA1 expression. Immunohistochemical analysis showed a higher expression of COX-2 and a lower expression of ANXA1 in gastric tumours than those in noncancerous tissues. This profile is similar to the noncancerous tissues (Gao et al. 2014).

#### ROLE OF ANXA1 AS A BIOMARKER IN TUMOR CELLS

ANXA1 has important roles in oncogenic events. It acts as a productive differentiation marker in head and neck cancer. Lower level of ANXA1 in esophageal squamous cell carcinoma (ESCC) and head and neck squamous cell carcinoma (HNSCC) are linked with deficiency in cellular differentiation and indicates bigger tumour size and lymph node metastasis in HNSCC (Pedrero et al. 2004). While, lower presence of ANXA1 found in cervical cancer acts as a biological marker in determining the development of invasive cervical cancer and control of cell death related growth factor (Prates et al. 2015). In cervical cancer, ANXA1 blocks cyclin D1 and affects cell morphology, reducing the cell proliferation (Alldridge & Bryant 2003).

It poses an immediate anti-proliferative effect on cells, and thus acts as tumour suppressor (Ang et al. 2009). Higher expression of D1 gene causes angiogenesis in ovarian cancer (Su et al. 2013).

Besides, the enhanced amount of this protein links with poor prognosis in prostate cancer (Ponz-Sarvisé et al. 2014). Furthermore, rise in ANXA1 level acts as a marker for poor survival rate and enhanced tumour recurrence in esophageal squamous cell carcinoma (ESCC). Therefore, patient showing the presence of ANXA1 in nucleus indicates a bad survival rate than that of without ANXA1 nuclear expression. In controlling cell proliferation, ANXA1 nuclear translocation plays a role. Nuclear localisation of ANXA1 is a vital occurrence in ESCC, providing a prognosis factor in human cancer (Lin et al. 2008). Therefore, higher or lower level of ANXA1 in ESCC cells may show the bad prognosis for this cancer.

#### IMPORTANCE OF ANXA1 IN IMMUNITY AGAINST INFECTION

An *in vivo* study was conducted on *Mycobacterium tuberculosis* (Mtb) CDC1551 strain of ANXA1 deficient mice and WT mice. After the injection of Mtb bacterial strain, the lungs and spleens of the mice were removed. From the spleen, the T-cells were isolated. Bone marrow derived macrophages were characterised. Bacterial load was found to higher in ANXA1 deficient mice than WT mice. It was significantly different from WT mice with  $p < 0.05$ . An ANXA1 deficient mouse poses bigger injury in lung and spleen as compared to wild type mice. The manifestation of inflammation is influenced by permeation of lymphocytes (Vanessa et al. 2015). TNF- $\alpha$  and IFN- $\gamma$  are involved in regulation of immunity against infection (Cruz et al. 2006).

#### BIAS ANALYSIS

From a total of 27 articles included in this study, it was found that five of them had potential to bias. These were due to reasons that some of the studies did not have a reliable and valid outcome. The remaining 22 articles had no risk to bias. Bias outcome was reported in Tables 3 and 4 (Supplemental data).

#### CONCLUSION

In conclusion, ANXA1 is found to cause apoptosis of neutrophils, inhibition of inflammatory mediators, inhibition of proinflammatory cytokines, stimulation of IL10, and blocking of NO synthesis. It is involved in cell growth, differentiation and proliferation. In addition, ANXA1 also acts as a marker in tumour cells and indicated the survival rate. Nuclear localisation of ANXA1 is a vital occurrence in OSCC providing as a



prognosis factor in human cancer. However, in cervical cancer, ANXA1 blocked cyclin D1. Therefore, reduced the cell proliferation. It possesses an immediate anti-proliferative effect on tumour suppressor cells. This study identified a potential research gap in the development of ANXA1 as a therapeutic agent in treating severe inflammation conditions and a tumour marker.

#### ACKNOWLEDGEMENTS

This study was funded by the Ministry of Higher Education, Malaysia for Fundamental Research Grant Scheme (FRGS), FRGS/1/2015/SKK09/UKM/02/3.

#### REFERENCES

- Adhami, V.M., Malik, A., Zaman, N., Sarfaraz, S., Siddiqui, I.A., Syed, D.N., Afaq, F., Pasha, F.S., Saleem, M. & Mukhtar, H. 2007. Combined inhibitory effects of green tea polyphenols and selective cyclooxygenase-2 inhibitors on the growth of human prostate cancer cells both *in vitro* and *in vivo*. *Clinical Cancer Research* 13(5): 1611-1619.
- Ahluwalia, A., Newbold, P., Brain, S.D. & Flower, R.J. 1995. Topical glucocorticoids inhibit neurogenic inflammation: Involvement of lipocortin 1. *European Journal of Pharmacology* 283(1-3): 193-198.
- Alldrige, L.C. & Bryant, C.E. 2003. Annexin 1 regulates cell proliferation by disruption of cell morphology and inhibition of cyclin D1 expression through sustained activation of the Erk1/2 mapk signal. *Experimental Cell Research* 290(1): 93-107.
- Ang, E.Z.F., Nguyen, H.T., Sim, H.L., Putti, T.C. & Lim, L.H. 2009. Annexin-1 regulates growth arrest induced by high levels of estrogen in MCF-7 breast cancer cells. *Molecular Cancer Research* 7(2): 266-274.
- Aziz, H., Hatah, E., Bakry, M.M. & Islahudin, F. 2016. How payment scheme affects patients' adherence to medications? A systematic review. *Patient Preference and Adherence* 10: 837-850.
- Cronstein, B.N. & Weissmann, G. 1993. The adhesion molecules of inflammation. *Arthritis & Rheumatology* 36(2): 147-157.
- Cruz, A., Khader, S.A., Torrado, E., Fraga, A., Pearl, J.E., Pedrosa, J., Cooper, A.M. & Castro, A.G. 2006. Cutting edge: IFN- $\gamma$  regulates the induction and expansion of IL-17-producing CD4 T cells during mycobacterial infection. *The Journal of Immunology* 177(3): 1416-1420.
- Ernst, S., Lange, C., Wilbers, A., Goebeler, V., Gerke, V. & Rescher, U. 2004. An annexin 1 N-terminal peptide activates leukocytes by triggering different members of the formyl peptide receptor family. *Journal of Immunology* 172(12): 7669-7676.
- Fadok, V.A., Bratton, D.L., Konowal, A., Freed, P.W., Westcott, J.Y. & Henson, P.M. 1998. Macrophages that have ingested apoptotic cells *in vitro* inhibit proinflammatory cytokine production through autocrine/paracrine mechanisms involving TGF- $\beta$ , PGE $_2$  and PAF. *Journal of Clinical Investigation* 101(4): 890.
- Ferlazzo, V., D'agostino, P., Milano, S., Caruso, R., Feo, S., Cillari, E. & Parente, L. 2003. Anti-inflammatory effects of annexin-1: Stimulation of IL-10 release and inhibition of nitric oxide synthesis. *International Immunopharmacology* 3(10-11): 1363-1369.
- Ferreira, S.H., Cunha, F.Q., Lorenzetti, B.B., Michelin, M.A., Perretti, M., Flower, R.J. & Poole, S. 1997. Role of lipocortin-1 in the anti-hyperalgesic actions of dexamethasone. *British Journal of Pharmacology* 121(5): 883-888.
- Flower, R.J. & Rothwell, N.J. 1994. Lipocortin-1: Cellular mechanisms and clinical relevance. *Trends in Pharmacological Sciences* 15(3): 71-76.
- Fox, S., Leitch, A.E., Duffin, R., Haslett, C. & Rossi, A.G. 2010. Neutrophil apoptosis: Relevance to the innate immune response and inflammatory disease. *Journal of Innate Immunity* 2(3): 216-227.
- Gao, Y., Chen, Y., Xu, D., Wang, J. & Yu, G. 2014. Differential expression of ANXA1 in benign human gastrointestinal tissues and cancers. *BMC Cancer* 14: 520.
- Gerke, V., Creutz, C.E. & Moss, S.E. 2005. Annexins: Linking Ca $^{2+}$  signalling to membrane dynamics. *Nature Reviews Molecular Cell Biology* 6(6): 449-461.
- Gibbs, L., Carollo, M.G., Damazo, A.S., Oliani, S.M. & Perretti, M. 2002. Time-dependent expression of annexin 1 in a model of chronic granulomatous inflammation. *Inflammation Research* 51(6): 300-306.
- Gimenes, A.D., Andrade, T.R., Mello, C.B., Ramos, L., Gil, C.D. & Oliani, S.M. 2015. Beneficial effect of annexin A1 in a model of experimental allergic conjunctivitis. *Experimental Eye Research* 134: 24-32.
- Hatah, E., Braund, R., Tordoff, J. & Duffull, S.B. 2014. A systematic review and meta-analysis of pharmacist-led fee-for-services medication review. *British Journal of Clinical Pharmacology* 77(1): 102-115.
- Hirata, F., Schiffmann, E., Venkatasubramanian, K., Salomon, D. & Axelrod, J. 1980. A phospholipase A2 inhibitory protein in rabbit neutrophils induced by glucocorticoids. *Proceedings of the National Academy of Sciences USA* 77(5): 2533-2536.
- Ker, R.F., Wang, X.T. & Pike, A. 2000. Fatigue quality of mammalian tendons. *Journal of Experimental Biology* 203(8): 1317-1327.
- Kosicka, A., Cunliffe, A.D., Mackenzie, R., Zariwala, M.G., Perretti, M., Flower, R.J. & Renshaw, D. 2013. Attenuation of plasma annexin A1 in human obesity. *The FASEB Journal* 27(1): 368-378.
- Lim, L.H., Solito, E., Russo-Marie, F., Flower, R.J. & Perretti, M. 1998. Promoting detachment of neutrophils adherent to murine postcapillary venules to control inflammation: Effect of lipocortin 1. *PNAS* 95(24): 14535-14539.
- Lima, K.M., Vago, J.P., Caux, T.R., Negreiros-Lima, G.L., Sugimoto, M.A., Tavares, L.P., Arribada, R.G., Carmo, A., Galvao, I., Costa, B.R.C., Soriani, F.M., Pinho, V., Solito, E., Perretti, M., Teixeira, M.M. & Sousa, L.P. 2017. The resolution of acute inflammation induced by cyclic amp is dependent on annexin A1. *Journal of Biological Chemistry* 292(33): 13758-13773.

- Lin, C.Y., Jeng, Y.M., Chou, H.Y., Hsu, H.C., Yuan, R.H., Chiang, C.P. & Kuo, M.Y.P. 2008. Nuclear localization of annexin A1 is a prognostic factor in oral squamous cell carcinoma. *Journal of Surgical Oncology* 97(6): 544-550.
- Lopresti, A.L. 2017. Cognitive behaviour therapy and inflammation: A systematic review of its relationship and the potential implications for the treatment of depression. *Australian & New Zealand Journal of Psychiatry* 51(6): 565-582.
- Lu, Q.Y., Jin, Y., Mao, J.T., Zhang, Z.F., Heber, D., Dubinett, S.M. & Rao, J. 2012. Green tea inhibits cyclooxygenase-2 in non-small cell lung cancer cells through the induction of annexin-1. *Biochemical and Biophysical Research Communications* 427(4): 725-730.
- Martin, G.R., Perretti, M., Flower, R.J. & Wallace, J.L. 2008. Annexin-1 modulates repair of gastric mucosal injury. *American Journal of Physiology-Gastrointestinal and Liver Physiology* 294(3): G764-769.
- Monastyrskaya, K., Babychuk, E.B., Draeger, A. & Burkhard, F.C. 2013. Down-regulation of annexin A1 in the urothelium decreases cell survival after bacterial toxin exposure. *Journal of Urology* 190(1): 325-333.
- Ng, F.S.P., Wong, K.Y., Guan, S.P., Mustafa, F.B., Kajiji, T.S., Bist, P., Biswas, S.K., Wong, W.S.F. & Lim, L.H.K. 2011. Annexin-1-deficient mice exhibit spontaneous airway hyperresponsiveness and exacerbated allergen-specific antibody responses in a mouse model of asthma. *Clinical & Experimental Allergy* 41: 1793-1803.
- Office of Health Assessment and Translation (OHAT). 2015. *Handbook for Conducting a Literature-Based Health*. [https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookjan2015\\_508.pdf](https://ntp.niehs.nih.gov/ntp/ohat/pubs/handbookjan2015_508.pdf). Accessed on 2 October 2017.
- Oliani, S.M., Damazo, A.S. & Perretti, M. 2002. Annexin 1 localisation in tissue eosinophils as detected by electron microscopy. *Mediators of Inflammation* 11(5): 287-292.
- Ortega-Gómez, A., Perretti, M. & Soehnlein, O. 2013. Resolution of inflammation: An integrated view. *EMBO Molecular Medicine* 5(5): 661-674.
- Patel, H.B., Kornerup, K.N., Sampaio, A.L.F., D'acquisto, F., Seed, M.P., Girol, A.P., Gray, M., Pitzalis, C., Oliani, S.M. & Perretti, M. 2012. The impact of endogenous annexin a1 on glucocorticoid control of inflammatory arthritis. *Annals of the Rheumatic Diseases* 71(11): 1872-1880.
- Pedrero, J.M.G., Fernandez, M.P., Morgan, R.O., Zapatero, A.H., Gonzalez, M.V., Nieto, C.S. & Rodrigo, J.P. 2004. Annexin A1 down-regulation in head and neck cancer is associated with epithelial differentiation status. *The American Journal of Pathology* 164(1): 73-79.
- Pei, L., Zhang, J., Zhao, F., Su, T., Wei, H., Tian, J., Li, M. & Shi, J. 2011. Annexin 1 exerts anti-nociceptive effects after peripheral inflammatory pain through formyl-peptide-receptor-like 1 in rat dorsal root ganglion. *British Journal of Anaesthesia* 107(6): 948-958.
- Ponz-Sarvisé, M., Castañón, E., Panizo-Santos, A., Redrado, M., López, I., Rosell, D., Gil-Aldea, I., Calvo, A., Nguewa, P.A. & Gil-Bazo, I. 2014. Differential tumor expression of inhibitor of differentiation-1 in prostate cancer patients with extreme clinical phenotypes and prognostic implications. *Clinical Genitourinary Cancer* 12(2): 87-93.
- Prates, J., Franco-Salla, G.B., Dinarte Dos Santos, A.R., Da Silva Jr., W.A., Da Cunha, B.R., Tajara, E.H., Oliani, S.M. & Rodrigues-Lisoni, F.C. 2015. Anxa1ac2-26 peptide reduces Id1 expression in cervical carcinoma cultures. *Gene* 570(2): 248-254.
- Probst-Cousin, S., Kowolik, D., Kuchelmeister, K., Kayser, C., Neundorfer, B. & Heuss, D. 2002. Expression of Annexin-1 in multiple sclerosis plaques. *Neuropathology & Applied Neurobiology* 28(4): 292-300.
- Rescher, U., Goebeler, V., Wilbers, A. & Gerke, V. 2006. Proteolytic cleavage of annexin 1 by human leukocyte elastase. *Biochimica et Biophysica Acta* 1763(11): 1320-1324.
- Rhee, H.J., Kim, G.Y., Huh, J.W., Kim, S.W. & Na, D.S. 2000. Annexin I is a stress protein induced by heat, oxidative stress and a sulfhydryl-reactive agent. *European Journal of Biochemistry* 267(11): 3220-3225.
- Ricciotti, E. & Fitzgerald, G.A. 2011. Prostaglandins and inflammation. *Arteriosclerosis Thrombosis and Vascular Biology* 31(5): 986-1000.
- Rodrigo, J.P., Garcia-Pedrero, J.M., Gonzalez, M.V., Fernandez, M.P., Suarez, C. & Herrero, A. 2004. Expression of annexin A1 in normal and chronically inflamed nasal mucosa. *Archives of Otolaryngology - Head & Neck Surgery* 130(2): 211-215.
- Soehnlein, O. & Lindbom, L. 2010. Phagocyte partnership during the onset and resolution of inflammation. *Nature Reviews Immunology* 10(6): 427-439.
- Su, Y., Gao, L., Teng, L., Wang, Y., Cui, J., Peng, S. & Fu, S. 2013. Id1 enhances human ovarian cancer endothelial progenitor cell angiogenesis via Pi3k/Akt and NF- $\kappa$ B/mmp-2 signaling pathways. *Journal of Translational Medicine* 11(1): 132.
- Vago, J.P., Nogueira, C.R., Tavares, L.P., Soriani, F.M., Lopes, F., Russo, R.C., Pinho, V., Teixeira, M.M. & Sousa, L.P. 2012. Annexin A1 modulates natural and glucocorticoid-induced resolution of inflammation by enhancing neutrophil apoptosis. *Journal of Leukocyte Biology* 92(2): 249-258.
- Vanessa, K.H., Julia, M.G., Wenwei, L., Michelle, A.L., Zarina, Z.R., Lina, L.H. & Sylvie, A. 2015. Absence of annexin A1 impairs host adaptive immunity against mycobacterium tuberculosis *in vivo*. *Immunobiology* 220(5): 614-623.
- Vergnolle, N., Pages, P., Guimbaud, R., Chaussade, S., Bueno, L., Escourrou, J. & Comera, C. 2004. Annexin 1 is secreted *in situ* during ulcerative colitis in humans. *Inflammatory Bowel Diseases* 10(5): 584-592.
- Vong, L., D'acquisto, F., Pederzoli-Ribeil, M., Lavagno, L., Flower, R.J., Witko-Sarsat, V. & Perretti, M. 2007. Annexin 1 cleavage in activated neutrophils a pivotal role for proteinase 3. *Journal of Biological Chemistry* 282(41): 29998-30004.
- Yang, Y.H., Hutchinson, P., Santos, L.L. & Morand, E.F. 1998. Glucocorticoid inhibition of adjuvant arthritis synovial macrophage nitric oxide production: Role of lipocortin 1. *Clinical & Experimental Immunology* 111(1): 117-122.

Yazid, S., Gardner, P.J., Carvalho, L., Chu, C.J., Flower, R.J., Solito, E., Lee, R.W., Ali, R.R. & Dick, A.D. 2015. Annexin-A1 restricts Th17 cells and attenuates the severity of autoimmune disease. *Journal of Autoimmunity* 58: 1-11.

Zhang, Z., Huang, L., Zhao, W. & Rigas, B. 2010. Annexin 1 induced by anti-inflammatory drugs binds to NF-kB and inhibits its activation: Anticancer effects *in vitro* and *in vivo*. *Cancer Research* 70(6): 2379-2388.

Drug and Herbal Research Centre  
Faculty of Pharmacy  
Universiti Kebangsaan Malaysia  
50300 Kuala Lumpur, Federal Territory  
Malaysia

\*Corresponding author; email: e\_kumolosasi@ukm.edu.my

Received: 23 April 2020

Accepted: 1 July 2020

## SUPPLEMENTAL DATA

TABLE 1. Summary of *in vivo* studies

Author (year)	Animal model	Treatment/ Chemical	Method	Results	Comment
Lim et al. (1998)	Male swiss albino mice	Zymosan	Mice were injected with zymosan and then treated with human recombinant ANXA1 and mimetic peptide Ac2-26  This protein was purified by fast protein liquid chromatography	Zymosan caused rise in cell adhesion and emigration  ANXA1 and mimetic peptide Ac2-26 caused detachment of leukocytes	ANXA1 blocks adherent neutrophils from going into the diapedesis and at the end detaches it from post capillary venule
Martin et al. (2008)	Male C57L6 mice	Ac2-26 and Boc2	Gastric ulcers were induced using acetic acid in the mice and western blot carried out to analyze ANXA1  Tissue samples incubated with ANXA1 polyclonal antibody  WT and ANXA1 deficient mice were treated with indomethacin to evaluate the severity	ANXA1 was expressed highly in ulcer induced mice. In non-ulcer induced mice, the level is low  Treatment upon Ac2-26 showed an increase in the healing of ulcer but showed the opposite when treated Boc-2	ANXA1 helps in the repair of gastric mucosal injury. Ac2-26 has improved healing
Gibbs et al. (2002)	Female TO mice	0.1% croton oil in Freund's complete adjuvant (CO/ FCA)	Tissues were extracted and used for examination of protein and mRNA  Western blotting was carried out to detect the protein Total RNA was isolated by electrophoresis. Hybridization was carried out	There was no difference between 37kDa and 33kDa proteins being expressed in inflamed tissue  The expression was increased. Through northern blotting, mRNA expression was determined	Infiltrate neutrophils are important in ANXA1 expression and its level is significantly increased during inflammation

			Blood samples were centrifuged. Detection on the localization of ANXA1 from the inflamed tissue	PMN and MNC were increased in inflamed tissue  Plasma corticosterone level was enhanced in animals with CO/FCA Sheep serum antibody was able to detect both 33kDa and 37kDa	
Yang et al. (1998)	Male Sprague Dawley rats	Dexamethasone	By injection, adjuvant arthritis (AA) induced inflammation in the mice. The synovial tissue was isolated from knee and hind foot. Through this tissue, synovial macrophages were obtained. Cells were treated with dexamethasone, LPS and anti- lipocortin-1. Nitric oxide released in macrophages was determined. Dexamethasone was added to the cells. Western blot was used to analyse the protein	Upon inflammation, NO is produced in the AA. When dexamethasone was given, it inhibited NO production  The synovial macrophages in AA showed a presence of lipocortin-1. This content was increased further with the administration of dexamethasone	LC1 is an important mediator of glucocorticoid to produce its anti-inflammatory actions upon synovial macrophages
Ahluwalia et al. (1995)	Male wistar rat	Betamethasone- 17-valerate	Skin of hind paw was treated with betamethasone- 17-valerate to determine the effect of steroid on treating oedema	Glucocorticoids blocked oedemaformation in rats. Treatment upon antiserum to lipocortin-1 has reversed the inhibitory effect by 29%	When glucocorticoid is applied upon any inflammatory condition, lipocortin-1 is induced on the cell surface
Oliani et al. (2002)	Male Sprague Dawley rats	Carragenin	Mice were treated with anti ANXA1 antibodies Localization of ANXA1 in tissue eosinophils was detected by electron microscope	After injecting carragenin, the expression of ANXA1 was increased in the cytoplasm  Percentage of ANXA1 found in eosinophil cytosol was majority whereas only small portion was found in plasma membrane	ANXA1 is found in a major portion in rat eosinophils



Pei et al. (2011)	Male Sprague Dawley rat	CFA	<p>CFA was injected into dorsal surface of rat's left hindpaw to induce inflammation.</p> <p>ANXA 12-26, BML-111 and Boc-1 peptides were used to determine the characterization of FPR2/ALX in anti-nociception of ANXA1</p> <p>Western blot analysis was carried out to determine the protein expression</p>	<p>Treatment with CFA has enhanced the expression of ANXA1</p> <p>ANXA1 released its anti nociceptive effect via FPR2/ALX</p> <p>ANXA1 and FPR2/ALX were found to be in satellite glial cells and neurons</p>	ANXA1 derived peptide is able to enhance the ANXA1 expression
Patel et al. (2012)	BALB/c wild type mice	Dexamethasone	<p>Mice were treated by K/BxN serum to induce inflammatory arthritis. Next, mice were given dexamethasone and some treated with ANXA1</p> <p>mRNA and protein expression in the ankle joint were observed by RT-PCR and immunohistochemistry</p>	<p>Treatment with dexamethasone has caused the induction of ANXA1 in tissue</p> <p>Mice with ANXA1 deficiency did not show improvement in inflammation whereas mice with presence of ANXA1 has shown improvement</p>	ANXA1 is vital to predict the efficacy of glucocorticoids
Jimenes et al. (2015)	Male wild type and ANXA1 knockout BALB/c mice	Ovalbumin	<p>Mice were pre treated with Ac2-26 and dexamethasone before ovalbumin was administered</p> <p>Severity of conjunctivitis was observed. Level of IgG was measured. Expression of ANXA1 in ocular tissues was measured by using western blot analysis</p>	<p>When OVA was given in WT and ANXA1 deficient mice, they presented with moderate to severe conjunctivitis</p> <p>Upon treatment with dexamethasone and Ac2-26 mimetic peptide, it reduced the severity</p> <p>Thus, the IgE level was increased when it was treated with OVA alone but the level then reduced when dexamethasone and Ac2-26 mimetic peptide were given</p>	ANXA1 controls the progression of conjunctivitis by inhibiting the activation of eosinophils, mast cells and neutrophils

Vanessa et al. (2015)	Balb/c WT and ANXA1 deficient mice	-	Mice were infected with mtb CD1551 bacterial strain. Lung and spleen were isolated. Histological analysis was conducted  Bone marrow derived dendritic cells (BMDCs) were obtained from mice and T cells were isolated  Infection of macrophage was identified by using bone marrow derived macrophages	Cells of infected lung and spleen of ANXA1 deficient mice showed an increase in bacterial load compared to WT mice  In ANXA1 deficient mice, inflammation was characterized by infiltration and aggregation of lymphocytes and macrophages  For WT mice, inflammation was more characterized and restricted to vascular associated focal lesions	Absence of lipocortin- 1 interferes with the adaptive immunity in the presence of bacteria
Yazid et al. (2015)	C57b1/6 and B10. RIII mice	Human recombinant ANXA1	Mice were administered with human recombinant ANXA1 Lens and retina were dissected and purified rat anti mouse CD16/CD 32 monoclonal antibody was added  Western blot analysis was carried out to assess protein. RNA detection was done by PCR	Mice with ANXA1 deficiency showed more retinal inflammation to that of wild type mice  ANXA1 deficient mice showed an increase in monocytes, neutrophils and macrophages  When this protein was present, it attenuated the severity of inflammation It also caused the alterations in T cell differentiation and proliferation	In a condition of enhanced autoimmune pathway, ANXA1 deficient mice possess an increase in Th17 and IL-17
Ferreira et al. (1997)	Male wistar rat	PGE2, carrageenin, bradykinin, TNF alpha, IL-1 beta, dopamine, dexamethasone, lipocortin-12-26	Rats were injected with PGE2, carrageenin, bradykinin, TNF alpha, IL-1 beta and dopamine to induce hyperalgesic effect  The rats were then injected with dexamethasone and lipocortin- 12-26	Hyperalgesic was induced upon administration of PGE2, carrageenin, bradykinin, TNF alpha, IL-1 beta, and dopamine  Dexamethasone induced lipocortin-1 thus inhibited the hyperalgesic effect	ANXA1 mediates its effect by the presence of dexamethasone and thus blocks the cytokines involved in hyperalgesic and COX-2

Ng et al. (2011)	ANXA1 deficient and WT mice	Ovalbumin	Mice were injected with ovalbumin. Airway resistance and lung compliance were measured	Total IgE, IgG2a and IgG2b levels were observed higher in ANXA1 deficient mice compared to WT mice	Lack in presence of ANXA1 leads to clinical changes in allergic asthma
Vago et al. (2012)	Male BALB/ c mice	Rolipram, Dexamethasone	Injection of lipopolysaccharides (LPS) into the mice and then pleural cavity cells were harvested. Rolipram and dexamethasone were given to mice. 4h LPS-challenged mice were given peptide Ac2-26 to lower inflammation. Cell death of leukocyte was evaluated morphologically. The products from cell lysis were prepared with incubation of primary antibody and analyzed by western blot	Neutrophil recruitment induced by LPS showed drop in ANXA1 expression  Rolipram reduced amount of neutrophil and increased cell death events. It also increased levels of ANXA1  ANXA1 is expressed during natural and drug-induced resolution of inflammation	During natural and GC- induced resolution of inflammation, ANXA1 plays an important role in inducing the cell death of inflammatory cells thus promoting apoptosis of neutrophils

TABLE 2. Summary of *in vitro* studies

Author (year)	Cell/ tissue model (cell type)	Method	Results	Comment
Rodrigo et al. (2004)	Mucosa tissue (from middle turbinate) in healthy patient and patient with perennial rhinitis. Nasal polyp from patient undergoing polypectomy	All tissue samples were treated in alcohol and embedded in paraffin  Immunohistochemical study was conducted to examine the ANXA1 expression	It was found that ANXA1 was expressed in a high level by the ciliated cells. It was found more in the apical surface and within cilia  The level of expression of ANXA1 was the same in epithelial cells and glands of normal and chronically inflamed nasal mucosa	Expression of ANXA1 in respiratory epithelium of nasal mucosa is related to cell type and differentiation and not being influenced by inflammatory disease

Rhee et al. (2000)	A459- human lung adenocarcinoma  HeLa- human cervix epitheloid carcinoma	Cells treated with heat, H2O2 and sodium arsenite  Protein amount was detected using western blot. ANXA1 mRNA expression was analyzed using northern blot. The total RNA was extracted	Rise in the level of ANXA11 and ANXA1 mRNA. The ANXA1 translocated from cytoplasm to nucleus and perinuclear region  In response to heat, H2O2 and arsenite, annexin 1 gene promoter region was induced	ANXA1 is a stress protein and may comprise of new group of protein
Ferlazzo et al. (2003)	Murine macrophage cell line J774	Cell line J774 was cultured. NO and cytokines were measured. IL-10 was determined by enzyme-linked immunosorbent assay (ELISA)  Cultures were treated with Ac2 -26 and incubated in LPS. RNA was isolated and analyzed by RT-PCR	Addition of human recombinant ANX-1 stimulated release of IL-10  Annexin 1 and its peptide derivative prevented the release of NO and IL-12 mRNA  It is due to the anti-inflammatory effect of annexin1 mediated by IL-10 thus inhibiting iNOS	AnnexinA1 stimulates the release of IL-10 which inhibits iNOS, NO, mRNA expression, IL-12 synthesis, reduce leukocyte migration
Lin et al. (2008)	SAS cell line	Presence of ANXA1 was determined immunohistochemically	Staining of ANXA1 in nucleus of OSCC patients showed a bad survival and it was a determiner of general survival rates ANXA1 nuclear mark observed in bigger tumor size and further metastasis indicating bad survival rate	Nuclear isolation of ANXA1 is a repeated event and vital prognostic factor in OSCC
Monastyrskaya et al. (2013)	UE cell line TEU-2 and human bronchial epithelial cell line	Cells were cultured and transepithelial electrical resistance was measured Quantitative reverse transcriptase- PCR and immunofluorescence was conducted to analyze the annexin expression in the bladder layers. RNA was isolated  Cells were evaluated on survival and differentiation after being exposed to pore forming bacterial toxin streptolysin O using microscope and alamar Blue assay	Expression of Annexin A1 was found high in UE. Due to the cell differentiation from TEU-2, this protein was up regulated  Cell survival was drastically reduced due to siRNA capture after bacterial toxin exposure and found in lower level in BPS patients	The reduction in this protein leads to declination of UE cell survival and causes progress of BPS



Rescher et al. (2006)	Human endothelial hybrid cell line EAhy.926	Blood leukocytes and endothelial cells were isolated. Purified Annexin A1 was inserted to leukocytes. Western blotting was done to determine Annexin A1 by adding anti-ANXA1 polyclonal antibody. It produced intact and cleaved parts of Annexin A1. Purified human recombinant Annexin A1 was cultured with recombinant HLE	Annexin A1 originally is of 37kDa but it was cleaved to 33kDa once treated with HLE thus promoting it as a substrate for HLE  When PMN was treated with human recombinant Annexin a1, it does not cause the degradation of Annexin A1	Annexin A1 acts as a substrate for HLE it helps in controlling leukocyte emigration to inflammatory sites via its cleaved peptide
Prates et al. (2015)	SiHa cell line (epidermoid carcinoma of cervix)	Ac2-26 peptide was added to the cell and served as experimental group and the morphology was seen. The control group was not manipulated  RNA was isolated from both groups. Rapid subtraction hybridization (RaSH) was conducted. Both strands of DNA was synthesized and obtained  The gene expression was analyzed by PCR	There was inhibition of cell proliferation in SiHa cell by Ac2-26. From the genes observed, 55 of them showed changes in expression when treated with peptide  Five of the up regulated genes were TPT1, LDHA, NCOA3, HIF1A, RAB13 and one gene was down regulated which was ID1. These five genes play role in tumorigenic and inflammatory process BMRP1B gene from the ID1 pathway also down regulated	This protein's peptide changes the proliferation in SiHa cells and serves it as a therapy for treating cervical carcinoma
Lu et al. (2012)	Lung cancer A549, NCI-H460 and NCI- H157, non neoplastic bronchial epithelial BEAS-2B cell line (ATCC)	Cell proliferation was conducted using those cells and treated green tea extract (GTE). All cells were treated with primary antibodies and immunoblot analysis was carried out. Total RNA was isolated using a chemical	By treating these cells with GTE, it blocked the A549 cell proliferation. It also caused increase in ANXA1 expression when treated with H157 Both H460 and H157 caused increase in ANXA1 and mRNA expression  When cells of A549 and H460 treated with GTE, the ANXA1 was increased while COX-2 and PLA2 was decreased  The level of COX-2 decreased due to the induction of ANXA1 production. When COX- 2 was up regulated, it over produces PGE2	GTE has shown its role in inflammation by expressing ANXA1 in different type of cancer cells and this avoids cancer and other inflammatory diseases

Ernst et al. (2004)	Human embryonic kidney (HEK) 293 cells	<p>The mimetic peptide of Annexin 1 (Ac1-25) was used. Extraction of blood granulocytes and monocytes were done</p> <p>Human granulocytes and monocytes were treated with Ac1-25</p> <p>By using PCR, cDNAs encoding FPR1 and FPR2 were achieved</p> <p>A chemotaxis assay was conducted for the movement of human monocytes, HEK 293 cells which express FPR, FPRL1 or FPRL2 or parental nmsa 293 cells</p>	<p>Annexin A1 exerted its anti inflammatory action through the N- terminal peptide Ac1-25 and acted as chemoattractant</p> <p>It activated human leukocytes and triggered chemotaxis by operating FPR and its family members.</p> <p>ANXA1 changed the morphology of neutrophils</p>	<p>ANXA1 acts as the ligand to FPR family and it is involved in controlling leukocyte movement into the inflamed area by activating or deactivating the FPR</p>
Hirata et al. (1980)	Rabbit peritoneal neutrophils	<p>Rabbit peritoneal neutrophils were prepared with glucocorticoids.</p> <p>The action of PLA2 activity was evaluated by the liberation of arachidonic acid from the cellular lipids. Bovine serum albumin was also used. The assay of porcine pancreatic PLA2 was done</p> <p>The effect of glucocorticoid was measured by observing the specific uptake of dexamethasone in neutrophils</p> <p>The protein was isolated from the neutrophils</p>	<p>When the neutrophil was treated with glucocorticoid, it lowered the release and synthesis of prostaglandins by blocking the pathway of PLA2</p> <p>The release of arachidonic acid was influenced by the different types of steroids used</p> <p>The percentage of inhibition varied</p> <p>Flucinoloneacetone was found to have the highest percentage of inhibition. Inhibitory effect occurred via cytoplasmic glucocorticoid receptors</p>	<p>This protein has the ability to block the PLA2 pathway thus reducing the chemotactic response of neutrophils</p>
Probst-Cousin et al. (2002)	Tissue samples from 10 patients with MS and five samples from patients without any neurological diseases	<p>Immunohistochemistry was done</p> <p>The expression of ANXA1 was detected</p>	<p>The presence of Annexin 1 was detected majorly in ependymal, subependymal, meninges, neurons and astrocytes</p> <p>This protein was not found in the white matter</p> <p>In MS cells, the presence of this protein was linked with the lesions where it is significantly reduced from active lesions through chronic stages</p>	<p>Presence of ANXA1 is linked with fresh MS lesions and it is stage reliant in the plaques</p>

Vergnolle et al. (2004)	Tissue samples from healthy patients, slight UC, moderate UC, severe UC inflamed and severe UC of healthy colon	All the tissue samples were obtained from the patients receiving anti-inflammatory therapy All the specimen were inoculated in culture medium and washed with normal saline to remove presence of ANXA1 in the cell surface  ANXA1 was then observed using western blotting	The MPO activity was observed Tissue samples from patient with severe UC of inflamed colon had the highest MPO activity whereas samples from severe UC with healthy colon had lowest MPO activity  ANXA1 was secreted in a high amount in severe UC condition whereas not detected in healthy samples	Annexin 1 is being released during the UC condition of inflamed colons
Ferreira et al. (1997)	J774 murine macrophages	The cells were cleaned with normal saline and added with LPS, lipocortin- 12-26 and dexamethasone	These cells blocked TNF- $\alpha$ which was produced by J774 cell	ANXA1 mediates its effect by the presence of dexamethasone and thus blocks the cytokines involved in hyperalgesic and COX-2 The anti- inflammatory role of lipocortin is within its peptide lipocortin- 12-26
Kosicka et al. (2013)	Blood samples from healthy and obese patients	ANXA1 level was measured in the human plasma Positive control was served with human neutrophils	Plasma ANXA1 was inversely proportional to the BMI indicating that as fat lump increases, the level of ANXA1 decreases	Diminished level of ANXA1 was present in overweight and obese patients

TABLE 3. Summary of risk of bias ratings for *in vivo* studies

Risk of bias/Author	Selection bias	Performance bias	Exclusion bias	Detection bias	Reporting bias
Vago et al. (2012)	+	+	+	+	+
Lim et al. (1998)	+	+	+	+	+
Martin et al. (2008)	+	+	+	+	+
Gibbs et al. (2002)	+	+	+	-	+
Yang et al. (1998)	+	+	+	+	+
Ahluwalia et al. (1995)	+	+	+	+	+
Oliani et al. (2002)	+	+	?	-	+
Pei et al. (2011)	+	+	+	+	+
Patel et al. (2012)	+	+	+	+	+
Gimenes et al. (2015)	+	+	+	+	+
Vanessa et al. (2015)	?	+	+	+	+
Yazid et al. (2015)	+	+	+	+	+
Ferreira et al. (1997)	+	+	+	+	+
Ng et al. (2011)	+	+	+	+	+

+ :No bias

- : Potential to bias

? : Unknown

TABLE 4. Risk of bias ratings for *in vitro* studies

Risk of bias/ Author	Selection bias	Performance bias	Exclusion bias	Detection bias	Reporting bias
Rodrigo et al. (2004)	+	+	?	-	+
Rhee et al. (2000)	+	+	+	+	+
Ferlazzo et al. (2003)	+	+	+	+	+
Monastyrskaya et al. (2013)	+	+	+	+	+
Rescher et al. (2006)	+	?	+	-	+
Prates et al. (2015)	+	+	+	+	+
Lu et al. (2012)	+	+	+	+	+
Ernst et al. (2004)	+	+	+	+	+
Hirata et al. (1980)	+	+	?	-	+
Probst-Cousin et al. (2002)	+	+	+	-	+
Vergnolle et al. (2004)	+	+	+	+	+
Kosicka et al. (2013)	+	+	+	+	+
Lin et al. (2008)	+	+	+	+	+
Ferreira et al. (1997)	+	+	+	+	+

+: No bias    -: Potential of bias    ?: Unknown